Nonalcoholic Steatohepatitis: Clinical Presentation, Diagnosis, and Treatment

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Abstract: Nonalcoholic fatty liver disease (NAFLD) is reaching epidemic proportions in our society and is the most common etiology for patients presenting with elevated liver enzymes. Given the significant numbers of patients presenting with NAFLD, it is important to distinguish between simple fatty liver and nonalcoholic steatohepatitis (NASH). Whereas simple fatty liver is thought to have a benign prognosis generally, NASH may progress to cirrhosis in a subset of patients. Performance of liver biopsies in all NAFLD patients is not feasible but recent studies have identified several clinical factors that may predict the patients at greatest risk for NASH and advanced fibrosis, and thus biopsy procedures may be confined to the patients meeting these criteria. Treatment remains focused on improving the underlying insulin resistance that is invariably present in the majority of patients. Diet and exercise remain the cornerstone of therapy, but insulin-sensitizing medication and other agents aimed at reducing oxidative stress or fibrosis may be considered as further studies demonstrating efficacy become available.

Nonalcoholic steatohepatitis (NASH) is a well-recognized form of chronic liver disease that is emerging as a major health concern in Western societies. Included within the broader diagnosis of nonalcoholic fatty liver disease (NAFLD), NASH is characterized by the presence of macrovesicular steatosis and predominantly lobular necroinflammation with or without pericellular and perisinusoidal fibrosis. NAFLD is generally considered to have a benign clinical course, but NASH may progress to cirrhosis,[1,2] and progression to hepatocellular carcinoma has also been reported.[3-5] The estimated prevalence of NAFLD ranges from 14% to 34% of the general population,[6-8] whereas NASH is thought to occur in approximately 3%,[9] with significantly greater prevalence in obese individuals.[10-13]

Keywords
Nonalcoholic fatty liver disease, nonalcoholic steatohepatitis, insulin resistance, obesity, metabolic syndrome

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Table 1. Components of the Metabolic Syndrome

- Abdominal obesity (waist circumference in men >40 in and in women >35 in)
- Triglycerides ≥150 mg/dL
- HDL cholesterol <40 mg/dL in men and <50 mg/dL in women
- Blood pressure ≥130/≥85 mm Hg
- Fasting glucose ≥110 mg/dL

Guidelines from the National Cholesterol Education Program (Adult Treatment Panel III).

HDL = high-density lipoprotein.

The obesity epidemic in our society is thought to have propagated the growing problem of NAFLD. The most recent National Health and Nutrition Examination Survey (NHANES) reveals that more than 30% of American adults are obese.[14] The metabolic syndrome, which includes obesity, diabetes mellitus, hypertension, and dyslipidemia (Table 1), is present in 25% of adults over 20 years of age and up to 45% of adults over the age of 50.[15] NAFLD is now considered to be the hepatic manifestation of this process, as both are integrally related to underlying insulin resistance. Consequently, it is incumbent upon healthcare providers to understand the clinical presentation of this disease; to attempt to correctly diagnose those with NASH, and to initiate a therapeutic plan targeted at correcting the underlying metabolic abnormalities in the patients. This review summarizes our current understanding of NAFLD as it pertains to clinical presentation, diagnosis, and treatment.

Clinical Presentation

Patients with NAFLD typically present in the fourth or fifth decade of life with mild elevations in liver aminotransferases, although the disease may also occur in childhood.[16] There appears to be an equal gender distribution, but females may have a greater risk of progression to advanced stages of disease.[16] Although ethnic variation exists, it has been suggested that there is a higher prevalence of NASH among white and Hispanic patients compared with African American patients.[17,18] Clinical conditions that are commonly found in NASH patients include those comprising the metabolic syndrome. Obesity is identified most commonly, with dyslipidemia, diabetes, and hypertension having a variable distribution among studies (Table 2).[1,19-24] However, a minority of patients with NAFLD will be of normal weight.[21] Most patients are asymptomatic but a few will present with fatigue or a vague, nondescript pain or discomfort in the right upper quadrant. Physical examination is typically significant for overweight or obesity but stigmata of chronic liver disease are usually absent, except in cases of clinically obvious advanced liver disease. Hepatomegaly occurs in approximately 10% of cases.

Liver enzymes, specifically alanine aminotransferase (ALT) and aspartate aminotransferase (AST), are mildly elevated with an ALT predominance and usually not exceeding 250 IU/L. The mean ALT and AST levels from a large cohort of biopsy-proven NASH patients were recently found to be 69 and 51 IU/L, respectively.[23] Some patients may have normal serum aminotransferases or only intermittent elevations on repeated studies, and evidence suggests that even normal liver enzymes do not exclude NASH or advanced stages of disease.[25] Alternatively, a small percentage of patients, possibly as many as 10%, may present with an isolated elevated alkaline phosphastase. These patients tend to be older women.[26] Auto-antibodies, such as antinuclear antibodies, may be found in up to one third of patients.[27] Serum iron studies are often abnormal and elevated ferritin levels can be seen in 40–58% of patients.[21,22,24] Indeed, evidence suggests that serum ferritin levels can be a marker of insulin resistance.[28] The association of hemachromatosis gene mutations and fibrosis with hepatic iron overload in NAFLD patients remains controversial.[24,29,30]

Diagnosis

The diagnosis of NASH begins with a thorough history and physical examination. Certain medications, clinical conditions, and surgical procedures may be associated with fatty liver and should be ascertained (Table 3).[19-24] Specific questioning as to the amount of alcohol ingested on a daily basis and over a lifetime should be performed. Evidence suggests that physicians obtain an accurate history in up to 90% of cases.[31] This is important as an arbitrary cut-off of two drinks per day has been proposed to distinguish purely nonalcoholic causes of fatty liver from alcohol-related steatosis. However, it is quite possible that patients may have a fatty liver or even steatohepatitis as a result of both processes occurring concomitantly.

Initial laboratory testing should include serologies for hepatitis B and C viruses, and a fasting iron panel, antinuclear antibody, anti–smooth muscle antibody, antimitochondrial antibody, and thyroid function testing. If the patient is less than 40 years of age, then a serum ceruloplasmin level should be obtained. A simple quantitative method to calculate insulin resistance can be performed by utilizing the homeostasis model assessment (HOMA) index.[32] HOMA is calculated by taking the product of the fasting insulin (μU/m) and fasting glucose (mmol/L) and dividing by 22.5. Thus, it is helpful to add these two blood tests to the initial screening laboratory data.

Table 2. Prevalence of Parameters of the Metabolic Syndrome in Nonalcoholic Steatohepatitis Patients

First Author	Obesity	Diabetes Mellitus	Dyslipidemia	Hypertension
Ludwig[19]	90%	25%	67%	15%
Powell[20]	95%	36%	62%	ND
Bacon[21]	39%	21%	21%	18%
Angulo[22]	60%	28%	27%	ND
Harrison[23]	75%	45%	ND	68%
Chitturi[24]	57%	29%	ND	ND

NASH = nonalcoholic steatohepatitis; ND = no data.

Table 3. Alternative Etiologies of Nonalcoholic Fatty Liver Disease

- Medications
  - Amiodarone
  - Perhexilene
  - Methotrexate
  - Tamoxifen
  - Corticosteroids
  - Highly active antiretroviral therapies
    - Stavudine
    - Protease inhibitors
- Metabolic disorders
  - Abetalipoproteinemia
  - Wilson's disease
  - Peroxisomal disorders
  - Lipodystrophy
- Surgical procedures/other
  - Jejunoileal bypass
- Gastroplexy
  - Industrial solvent exposure
  - Celiac sprue
  - Total parenteral nutrition

Imaging studies are also routinely performed as part of the diagnostic evaluation. The two most commonly employed imaging modalities are ultrasound and computed tomography (CT). Ultrasound is less expensive and usually easier to obtain than CT. Typical sonographic findings include a hyperechoic liver with blurring of the vascular margins.[33] CT scans are more expensive, but somewhat more specific at detecting fatty liver. Unfortunately, the positive predictive value of these two studies is not ideal, ranging from 62% to 76%.[34,35] Magnetic resonance spectroscopy has been used to study intrahepatocellular lipids and fibrosis with some success, but has not been studied adequately as a screening tool.[36] Unfortunately, at the present time, no imaging study can accurately distinguish simple fatty liver from NASH.

The only way to distinguish simple fatty liver from NASH is by obtaining liver histology. A new scoring system has recently been proposed by Kleiner and associates[37] to diagnose NASH by standard liver biopsy technique. The NASH activity score, or NAS, employs the histologic features of steatosis, lobular inflammation, and ballooning. Steatosis and lobular inflammation are graded 0–3 based on severity, whereas ballooning is graded 0–2. The NAS is the unweighted sum of these collective scores. A NAS of 5 or higher correlates with a diagnosis of NASH whereas a NAS of less than 3 is not consistent with this diagnosis. Fibrosis is not included in the diagnostic paradigm. Although it appears that consensus on a histologic diagnosis is forming among hepatopathologists, several limitations to liver biopsy still exist and subsequently the role of liver biopsy in this disease is still debated.

Some clinicians argue that liver biopsy should be performed on all patients with suspected fatty liver disease to help differentiate NAFLD from NASH. Liver biopsy results allow physicians to recognize a potentially harmful disease and may have a psychological impact on patients that could encourage them to enact lifestyle changes, which may improve or resolve the condition. Clinicians who oppose liver biopsy in patients referred for abnormal liver function tests point out the potential risks of the procedure, the sheer volume of patients presenting with fatty liver, the expense of the procedure, and the fact that liver biopsy is not suitable for repetitive evaluation. Perhaps the most compelling argument against liver biopsy is the sampling variability that exists in patients with NAFLD. Ratziu and colleagues[38] recently demonstrated that on sequential biopsies obtained via the same entry point, there was a significant rate of discordance between the two biopsy samples in all grading and staging categories, except for the amount of steatosis.

Given the inherent limitations of liver biopsy, investigators are currently searching for noninvasive ways of distinguishing simple fatty liver from NASH and mild NASH from more advanced stages of disease in order to select for liver biopsy those patients at greatest risk for disease progression. Several studies have identified clinical and laboratory characteristics that may be used in a model

Table 4. Risk Factors for Severe (Stage 3–4) Hepatic Fibrosis in Nonalcoholic Steatohepatitis Patients

Study	Described Risk Factors
Angulo et al[22]	Age, obesity, DM, AST/ALT ratio
Marceau et al[11]	Age, steatosis, fasting blood sugar, WHR, BMI, DM
Garcia-Monson et al[10]	Age, steatosis, inflammation grade
Ratziu et al[41]	Age, BMI, ALT, triglycerides, inflammation grade
Dixon et al[12]	Hypertension, ALT, C-peptide, insulin resistance
Chitturi et al[24]	Female gender, DM, inflammation grade
Harrison et al[23]	Age, diabetes, female gender, AST/ALT ratio

ALT = alanine aminotransferase; AST = aspartate aminotransferase; BMI = body mass index; DM = diabetes mellitus; WHR = waist to hip ratio.

to differentiate NAFLD from NASH. Dixon and colleagues[12] retrospectively studied a group of obese patients undergoing bariatric surgery who had liver biopsies at the time of the procedure. They identified hypertension, an ALT level greater than 40 IU/L, and insulin resistance (HOMA score > 5.0) as factors that were predictive of NASH. When combined, these variables comprise the high-affinity insulin resistance (HAIR) score, with a sensitivity and specificity of 80% and 85%, respectively, for the diagnosis of NASH. Sumida and colleagues[39] measured thioredoxin, a stress-inducible protein, and ferritin levels in a small group of NAFLD patients and noted that these biomarkers were significantly more elevated in patients with NASH compared with those patients with simple fatty liver as well as healthy controls. The adipocytokine adiponectin was recently correlated with severity of NAFLD and found to be significantly lower in patients with NASH.[40] This same study showed that NASH patients tend to have a higher degree of insulin resistance than patients with simple fatty liver. Unfortunately, these results have not yet been validated prospectively in a separate cohort of patients, thus limiting their utility at the present time.

Several studies have retrospectively evaluated independent predictors of severe hepatic fibrosis in patients with NASH, with varying results (Table 4).[10-12,22-24,41] The majority of these studies were small and used a variety of histologic scoring systems. Utilizing data obtained from multivariate analysis, Angulo and colleagues[22] identified age of 45 years or older, obesity, diabetes mellitus, and an AST/ALT ratio greater than 1 as factors that distinguish NASH patients with stage 3–4 fibrosis. Utilizing four clinical variables identified on multivariate analysis, Ratziu and colleagues[41] created a model combining body mass index (BMI) of 28 or higher, age of 50 years or older, ALT levels measuring twice normal or higher, and triglyceride levels of 1.7 mmol/L or higher (BAAT). Each clinical variable was given a score of 0 or 1 with total scores ranging from 0 to 4. An overall score of 0 or 1 was associated with a sensitivity and specificity of 100% and 47% (negative predictive value 100%), respectively, for the absence of septal fibrosis. When all four variables are present (a score of 4) the specificity increased to 100% for detection of septal fibrosis, but the sensitivity decreased to 14%. Further work by Harrison and colleagues[23] in a group of 501 biopsy-proven NASH patients demonstrated on multivariate analysis that female gender, diabetes mellitus, AST/ALT ratio, and age of over 50 were independent predictors of advanced fibrosis.

Recent work has focused on the development of serologic markers of fibrosis, alone and in combination with clinical markers as mentioned above, for the detection of advanced fibrosis. Compared to the treatment of hepatitis C, where extensive data are available for noninvasive combination tests that include fibrosis markers as well as biochemical and clinical data, very little published data exist for NAFLD patients. Sakugawa and colleagues[42] studied type VI collagen 7S domain and hyaluronic acid in a group of 112 NAFLD patients. Both markers were independently associated with the presence of severe fibrosis. Laine and associates[43] developed a predictive index of significant fibrosis in a group of 173 patients with elevated liver enzymes and evidence of the metabolic syndrome. A hyaluronic acid level greater than 35 μg/L was not associated with any cases of significant fibrosis. Furthermore, among patients with a hyaluronic acid level greater than 35 μg/L, further exclusion of patients with stage 3 or 4 fibrosis was made when the carbohydrate–deficient transferrin/transferrin ratio was less than 0.9. Further analysis of various fibrosis models, such as those previously mentioned and those currently being developed for chronic hepatitis C, is warranted prior to incorporation of these tests into clinical practice.

Not all patients presenting with clinical evidence of NAFLD require a liver biopsy. The majority of patients will have simple fatty liver or mild NASH and thus are thought to be at little risk for progression of disease. However, it is imperative that patients with more severe steatohepatitis be detected early as evidence suggests that one third of these patients will die from liver-related causes or progress to hepatocellular carcinoma within 5 years.[44] Therefore, we suggest that patients who are obese, over 50 years of age, have coexistent insulin resistance or diabe-

tes, and who have AST/ALT ratios that exceed 0.8 should be offered a liver biopsy to further define their disease severity. If found to have more advanced stages of NASH, these patients should be counseled appropriately on their risk for disease progression and an aggressive therapeutic plan should be initiated.

Treatment

The management of patients with NASH can be challenging. Well-defined treatment modalities remain limited at the present time. Current data consist mainly of small, uncontrolled trials. However, it is clear that efforts should be made to correct the underlying metabolic syndrome that is invariably present in the vast majority of cases. This includes improvement of underlying insulin sensitivity, which may be achieved through recommended lifestyle changes that encourage dietary modifications and increase aerobic exercise, ultimately resulting in weight loss. This paradigm could potentially be augmented by the initiation of adjunctive pharmacologic therapy to assist with weight loss and reduce insulin resistance in patients not attaining therapeutic goals with diet and exercise alone or in patients with evidence of advanced disease. It is also imperative to avoid factors that promote progression of disease, including alcohol consumption or drug therapy with tamoxifen, amiodarone, diltiazem, or corticosteroids, which may lead to further fatty liver deposition.

Weight Loss

Obesity, and more specifically central adiposity, is a principal factor in the metabolic syndrome[45] and the most common clinical variable found in patients with NASH. Weight reduction leads to improved insulin sensitivity and therefore should be an initial focus in the management of patients with NASH. Gradual weight loss, achieving at least a 10% reduction in weight, appears to be effective in improving serum aminotransferase levels as well as underlying histology of hepatic steatosis, inflammation, and fibrosis.[46,47] Rapid weight loss through severe caloric restriction or gastroplasty may lead to increased portal or lobular inflammation.[48-50] Options to achieve weight reduction include diet, exercise, pharmacotherapy, and surgery.

Diet The goal of any diet should be to induce a negative net caloric intake compared with expenditure. While no well-established diets have been specifically tested in patients with NASH, evidence suggests that NAFLD patients consuming high-carbohydrate diets were more likely to have inflammation on liver biopsy.[51] Therefore, it appears that diets based on a lower processed carbohydrate platform may be beneficial in NASH patients. In fact, two studies following a decreased carbohydrate diet have demonstrated improved lipid profiles in overweight patients.[52,53] Huang and colleagues[54] recently demonstrated that a diet designed to reduce insulin resistance (40–45% of calories from mainly complex carbohydrates, 35–40% of calories from monounsaturated and polyunsaturated fats, and 15–20% of calories from protein) resulted in histopathologic improvement in 9 of 15 NASH patients who completed 1 year of intense dietary intervention with a mean weight loss of 2.9 kilograms. Another pilot study in 8 obese patients with type 2 diabetes showed that a moderately hypocaloric very low fat diet (3%) resulted in normalization of fasting plasma glucose concentrations, improved insulin sensitivity, and decreased intrahepatic lipid content.[55] Until specific dietary platforms are studied in NASH patients, the use of a heart-healthy, low–processed carbohydrate diet to produce a caloric deficit of 500–1,000 calories/day is recommended in overweight or obese individuals.

Exercise The benefits of exercise have been well described in reference to insulin resistance,[56,57] hypertension,[58] and serum lipid profiles.[59] Animal models have demonstrated that moderate exercise via treadmill 5 days per week prevented the development of fatty liver and resulted in decreased adiposity, nonesterified fatty acids, and leptin concentrations even when eating a high-fat diet.[60,61] The effect of exercise on NASH has not been directly evaluated. However, a small randomized study by Ueno and colleagues[62] assessed the benefits of walking and jogging in association with a 25 cal/kg per day diet in 13 patients with biopsy-proven fatty liver over a 3-month period, compared with 12 controls. The study patients began by walking 3,000 steps per day for 3 days with the addition of 500 steps every 3 days to achieve an ultimate goal of 10,000 steps daily. The study patients were then advanced to jogging for 20 minutes twice daily. Within the treatment group, BMI decreased over the 3-month period, as did serum aminotransferase, total cholesterol, and glucose levels. Repeat liver biopsy revealed a significant improvement in hepatic steatosis in the treatment group at 3 months. Patients with chronic liver disease and evidence of fatty liver who are able to maintain weight reduction via diet and exercise have sustained improvement in ALT levels, and fasting insulin. This improvement may be seen with as little as 4–5% body weight reduction.[63]

Pharmacotherapy Medical therapy for weight loss in overweight and obese patients is an area of active investigation, although only a few small studies in NASH patients exist. Orlistat (Xenical, Roche), a reversible inhibitor of gastric and pancreatic lipases, is currently approved for weight loss, and 6–12 months of daily therapy have demonstrated 5–10% overall total body weight reductions.[64] Small pilot

studies and case series conducted in NASH patients have demonstrated significant improvements in serum aminotransferases, hepatic steatosis, necroinflammatory activity, and fibrosis.[65,66] Most patients are able to tolerate this medication but some discontinue orlistat due to persistent loose stools or tenesmus. Sibutramine (Meridia, Abbott), a serotonin reuptake antagonist, is another pharmacologic agent that leads to an average weight loss of 5–8%.[67] One small study of 13 Turkish patients with NASH demonstrated that 6 months of sibutramine treatment resulted in a weight reduction of 10.2% and improvement in insulin resistance, serum aminotransferase levels, and ultrasound evidence of fatty liver disease.[68] However, no follow-up liver biopsies were obtained.

Surgery Bariatric surgery is gaining in popularity as a therapeutic option for patients with morbid obesity (BMI > 40 kg/m^2 or > 35 kg/m^2 coupled with obesity-associated comorbidities). Although earlier surgical procedures such as jejuno-ileal bypass were shown to induce or worsen NASH, more recent bariatric surgical procedures such as the laparoscopic gastric banding and roux-en-y gastric bypass appear to be beneficial. Dixon and colleagues[69] obtained repeat liver biopsies in 23 obese Australian patients with NASH 25.6 months after undergoing laparoscopic adjustable gastric banding for weight loss. Patients lost a mean of 34 kilograms, and significant improvements were seen in steatosis, necroinflammation, and fibrosis. Complete histopathologic resolution of NASH was found in 82% of patients. More recently, Barker and colleagues[70] demonstrated a similar improvement in histopathology in 19 obese American patients who underwent roux-en-y gastric bypass surgery and had biopsy-proven NASH at the time of surgery. Follow-up liver biopsies were obtained at a mean of 21.4 months after surgery. A mean weight loss of 52.4 kilograms was achieved. Similar to Dixon and colleagues, 89% of patients demonstrated complete histopathologic resolution of NASH. Bariatric surgery appears to be an effective therapeutic option for morbidly obese patients with NASH, although larger trials are eagerly awaited to confirm these early results.

Insulin-sensitizing Pharmacotherapy

The use of insulin-sensitizing medication to augment the effects of diet and exercise is gaining momentum. Several small pilot studies have assessed the benefits of the thiazolidinediones (TZDs; rosiglitazone [Avandia, GlaxoSmithKline] and pioglitazone [Actos, Takeda/Eli Lilly) and the biguanide metformin in the treatment of NASH.

The TZDs act as ligands for the peroxisomal proliferators–activated receptor-γ (PPAR-γ) class of nuclear transcription factors, which are largely concentrated in adipose tissue[61] and appear to improve insulin sensitivity via decreased hepatic glucose production and enhanced glucose disposal in muscle. Two pilot trials with pioglitazone have been published to date.[71,72] One study treated 18 adult nondiabetic NASH patients with 30 mg of pioglitazone daily for 48 weeks in a nonrandomized, open-label fashion.[71] Improvement in insulin sensitivity, serum aminotransferase levels, necroinflammation, steatosis, and fibrosis was seen in the majority of patients. A subsequent small, prospective, randomized trial of vitamin E 400 IU daily versus pioglitazone 30 mg plus vitamin E 400 IU daily for 48 weeks was also completed in 18 patients.[72] The patients who received combination therapy had significant improvement in insulin sensitivity, steatosis, cytologic ballooning, and pericellular fibrosis. Rosiglitazone has also been evaluated in an open-label trial of 4 mg twice daily for 48 weeks in 30 patients with biopsy-proven NASH.[73] Insulin sensitivity and ALT levels improved significantly with follow-up liver biopsies demonstrating significantly decreased necroinflammatory activity and improved perisinusoidal fibrosis. More recently, a small, randomized, double-blind, placebo-controlled, multicenter clinical trial with pioglitazone in 40 biopsy-proven NASH patients appears to confirm the beneficial effects of TZDs in patients with this disease.[74] Concerns over hepatotoxicity related to these medications remains in light of the previous experience with troglitazone. Among the four small studies described above, two patients discontinued medication due to rising aminotransferase levels.[72,73] Clearly, larger trials are needed to confirm both the efficacy and safety of TZDs.

Metformin is another insulin-sensitizing medication that has been studied in animal models of fatty liver disease and in a few human pilot trails. This agent improves insulin sensitivity through decreased hepatic glucose and triglyceride production. In mice, metformin has been shown to improve liver steatosis.[75] An open-label trial of metformin 20 mg/kg for 1 year in 15 NAFLD patients demonstrated improvement in serum aminotransferase levels and insulin sensitivity in the first 3 months but the serum aminotransferases subsequently returned to baseline levels and insulin sensitivity remained steady without further improvement. Histopathologic follow-up in 10 patients demonstrated minimal improvement overall.[76] Another study in 36 NASH patients randomized to metformin 850 mg twice daily plus diet versus diet alone for 6 months demonstrated improved serum aminotransferase levels and insulin sensitivity. However, no significant difference between groups was seen on follow-up liver biopsy.[77] An open-label randomized trial of metformin 2 g daily for 12 months versus either vitamin E 800 IU daily or a prescriptive weight-reducing diet in 55 patients showed that while liver enzymes improved in all groups, the improvement was more pronounced in

the metformin group.[78] Furthermore, in a small subset of those receiving metformin, a follow-up liver biopsy demonstrated improvement in steatosis, necroinflammation, and fibrosis. Unfortunately, no placebo-controlled trials with metformin have been published, limiting the enthusiasm for this medication in the treatment of NASH.

Antioxidant Pharmacotherapy

Chronic oxidative stress has been proposed as one of the pathophysiologic mechanisms for development of NASH. Hepatocyte injury may occur through oxidation of cytotoxic free fatty acids and upregulation of proinflammatory cytokines leading to depletion of intracellular antioxidants.[16] Using this principle, various therapies aimed at reducing oxidative stress have been studied using vitamin E, vitamin C, betaine, and therapeutic phlebotomy.

Vitamin E Vitamin E (alpha-tocopherol) has been shown in animal models to reduce oxidative stress and improve hepatic glutathione levels.[9,80] It has been evaluated in NASH patients as a single agent, in combination with vitamin C, and in combination with pioglitazone.

The first human trial of vitamin E in NAFLD was in a pediatric group of patients. These children were obese, had elevated serum aminotransferase levels, and had a presumed diagnosis of NAFLD. All patients were administered vitamin E 400–1,200 IU/day for approximately 5 months and had a subsequent improvement in serum aminotransferase levels.[81] Another small nonrandomized study in adult NASH patients treated with vitamin E 300 mg daily for one year demonstrated improvement in inflammation and fibrosis on repeat liver biopsy.[82] Vitamin E has also been evaluated in conjunction with vitamin C in NASH patients. A group of biopsy-proven NASH patients were randomized to vitamin E (1,000 IU/day) plus vitamin C (1,000 mg/day) or placebo for 6 months.[83] The patients showed no improvement in serum aminotransferase levels but repeat liver biopsies at the end of the trial demonstrated decreased fibrosis within the vitamin group (especially in patients with diabetes). More recently, a small randomized study assessed the efficacy of vitamin E alone (400 IU daily) compared with vitamin E (400 IU daily) and pioglitazone for 48 weeks.[72] Vitamin E decreased hepatic steatosis on follow-up liver biopsy, but the combination of vitamin E plus pioglitazone produced an even greater decrease in steatosis and improved fibrosis.

Betaine Betaine (trimethylglycine), a readily available nutritional supplement, is a component of the metabolic cycle of methionine, which allows for the formation of S-adenosyl-methionine and the methylation of toxic homocysteine metabolites. In vitro evidence suggests that betaine protects hepatocytes from apoptotic injury.[84] A single small, nonrandomized study of 10 biopsy-proven NASH patients treated with betaine has been published.[85] Patients were treated with 20 g daily of a betaine anhydrous solution for 1 year and repeat liver biopsies were obtained. Seven patients completed the study. Normalization or improvement in serum aminotransferase levels was seen in the majority of patients and significant improvement in steatosis, necroinflammatory activity, and fibrosis was seen on follow-up liver biopsy.

Therapeutic Phlebotomy Serum iron indices have been documented to be abnormal in up to 58% of patients.[24] Increased levels of iron within the liver parenchyma have been associated with increased oxidative stress. Therapeutic phlebotomy to near iron deficiency has been studied in small pilot trials with improvement in serum aminotransferase levels[86,87] and insulin sensitivity.[87] Unfortunately, correlative histopathologic improvement is not available as follow-up liver biopsies were not obtained.

Other Treatment Options

Angiotensin II Receptor Antagonists Hepatic stellate cell (HSC) activation is thought to be important in fibrosis progression in NASH patients. Once activated, normally quiescent HSCs express components of the renin-angiotensin system to include synthesis of the peptide angiotensin II.[88] This peptide has been shown to stimulate production of both tissue inhibitor of metalloproteinase-1[89] and vascular endothelial growth factor,[90] (two proteins involved in hepatic fibrogenesis), and is a major regulator of liver fibrosis.[91] Hepatic fibrosis is attenuated in animal models through treatment with angiotensin II type-1 receptor blockers or angiotensin-converting enzyme inhibitors.[91-93] Similarly, a small uncontrolled trial of losartan, a selective angiotensin II type-1 receptor antagonist, in 7 NASH patients, demonstrated significant improvement in serum aminotransferase levels, as well as serologic markers of hepatic fibrosis including hyaluronic acid, type IV collagen, and procollagen III N-terminal propeptide, although no improvement was seen in insulin resistance.[94] Repeat liver biopsies demonstrated decreased hepatic necroinflammation and fibrosis in the majority of cases.

Ursodeoxycholic Acid The precise mechanism of ursodeoxycholic acid (UDCA) is unknown, but evidence suggests that UDCA may help to reduce oxidative stress at the mitochondrial membrane level, ultimately resulting in decreased cellular apoptosis and necrosis. Early pilot trials of UDCA in NASH patients demonstrated improvement in serum aminotransferase levels.[95-97] However, a recent large prospective, double-blind, randomized, controlled

trial showed no improvement in serum aminotransferase levels, steatosis, necroinflammatory activity, or fibrosis.[98]

Pentoxifylline Tumor necrosis factor (TNF)-α is elevated in NASH patients and appears to contribute to inflammatory, apoptotic, and fibrogenic processes within the liver.[99] Pentoxifylline is a methylzanthine compound that inhibits TNF-α production.[100] A recent small pilot trial in 20 NASH patients treated with pentoxifylline (400 mg four times daily) for 12 months was performed.[101] Although serum aminotransferase levels improved, there was a high rate of gastrointestinal side effects and no follow-up histopathology was obtained.

Conclusion

A common and frequently underdiagnosed cause of liver disease, NAFLD is usually identified incidentally through the presence of fatty liver on imaging or mild serum aminotransferase abnormalities found on routine physical examinations or prior to initiation of lipid-lowering pharmacotherapy. The distinction between simple fatty liver and NASH with moderate to advanced fibrosis is important. Liver biopsy is still required to differentiate simple fatty liver from NASH. Given the inherent problems with liver biopsy as a diagnostic option, interest has focused on developing simple, noninvasive methods to diagnose NASH and NASH with advanced fibrosis. No current methods have been validated in prospective fashion, but several factors seem to predict the patients at greatest risk for more advanced disease. Liver biopsy is recommended for patients meeting these criteria.

Therapeutic options remain focused on improving the underlying insulin resistance that is invariably present in the majority of patients. The initiation of a heart-healthy diet, low in processed carbohydrates, to produce a caloric deficit of 500–1,000 calories/day is recommended in overweight or obese individuals, until studies of formal dietary programs can be undertaken in NASH patients to establish efficacy. Exercise as an adjunct to diet is also recommended, given the beneficial effects on insulin sensitivity seen in diabetic patients. However, data for NAFLD patients placed on specific exercise programs are limited. An exercise routine that focuses on aerobic activity for 30 minutes daily 5 days a week seems reasonable. Bariatric surgery appears to be effective in morbidly obese patients with NASH. Pharmacologic therapy in the form of insulin-sensitizing medication has been an area of intense investigation and the future looks very promising for its use as adjunctive therapy to diet and exercise. Further study with these agents, as well as weight-loss medication, specific antioxidants, and medications that may improve fibrosis (eg, angiotensin II receptor antagonists) is needed prior to adding them to our therapeutic armamentarium. However, in patients with advanced disease, who are ineligible for clinical trials, consideration for the use of these experimental agents should be made.

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